Transgenic Dairy Cattle: Genetic Engineering on a Large Scale

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ABSTRACT

Amid the explosion of fundamental knowledge generated from transgenic animal models, a small group of scientists has been producing transgenic livestock with goals of improving animal production efficiency and generating new products. The ability to modify mammary-specific genes provides an opportunity to pursue several distinctly different avenues of research. The objective of the emerging gene "pharming" industry is to produce pharmaceuticals for treating human diseases. It is argued that mammary glands are an ideal site for producing complex bioactive proteins that can be cost effectively harvested and purified. Consequently, during the past decade, approximately a dozen companies have been created to capture the US market for pharmaceuticals produced from transgenic bioreactors estimated at $3 billion annually. Several products produced in this way are now in human clinical trials. Another research direction, which has been widely discussed but has received less attention in the laboratory, is genetic engineering of the bovine mammary gland to alter the composition of milk destined for human consumption. Proposals include increasing or altering endogenous proteins, decreasing fat, and altering milk composition to resemble that of human milk. Initial studies using transgenic mice to investigate the feasibility of enhancing manufacturing properties of milk have been encouraging. The potential profitability of gene "pharming" seems clear, as do the benefits of transgenic cows producing milk that has been optimized for food products. To take full advantage of enhanced milk, it may be desirable to restructure the method by which dairy producers are compensated. However, the cost of producing functional transgenic cattle will remain a severe limitation to realizing the potential of transgenic cattle until inefficiencies of transgenic technology are overcome. These inefficiencies include low rates of gene integration, poor embryo survival, and unpredictable transgene behavior.

(**Key words**: transgenic, bioreactor, genetic engineering, milk composition)

Abbreviation key: **CoA** = coenzyme A, **hGH** = human growth hormone.

INTRODUCTION

In 1987, Lothar Hennighausen and Heiner Westphal at the National Institutes of Health, in collaboration with Katy Gordon and her colleagues at Integrated Genetics, started a mini-revolution when they reported that a pharmaceutical could be produced in mammary glands of transgenic animals (28). Within 5 yr of their report, a new industry was formed, sponsored with venture capital. The remarkable speed with which transgenic animal bioreactor technology moved from laboratories to industry attests to the perceived potential value of this approach for producing pharmaceuticals. The dairy industry has not embraced transgenic technology with the same enthusiasm as the pharmaceutical industry. In this review, we explain why and try to identify productive areas of research that could provide supporting justification for the dairy industry to consider the transgenic approach as a practical means to enhance the genetic potential of dairy cattle.

Goals for Genetic Engineering of Mammary Glands

Commercial interests have fueled research on modifying the genetic control of mammary glands for the purpose of producing pharmaceutical proteins in milk. However, this technology also offers the opportunity to alter the composition of bovine milk destined for the dairy industry. A number of excellent reviews (38, 49, 60, 82, 84) have been written on the topic. The authors of those reviews have suggested strategies for changing milk composition to enhance cheese yield, to reduce the energetic cost of milk production,

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TABLE 1. Milestones in transgenic animal bioreactor technology.

Year	Accomplishment	Reference
1980	Transgenic animal produced by pronuclear microinjection.	(27)
1982	Fusion gene introduced into transgenic mice.	(10)
1987	Expression of pharmaceutical directed to the mammary gland of transgenic mice.	(28)
1988	First transgenic bioreactor farm animals produced (sheep).	(69)
1991	Mammary gland specific transgene expressed in goats.	(22)
1991	Mammary gland specific transgene expressed in pigs.	(79)
1991	Transgenic bull produced with mammary gland specific gene construct.	(43)
1994	Bioreactor products being tested in animal models (preclinical testing).	. . .[1]

[1]Henryk Lubon (1994, personal communication).

and to reduce the microbial load in milk. One of the most ambitious schemes that has been proposed is to alter the composition of bovine milk so that it resembles human milk by eliminating some bovine milk protein genes and replacing others with coding sequences derived from human genes. The resulting milk, which would more closely resemble human breast milk, would be used to supplement or replace infant formula.

Economic Considerations

Genetic improvements in livestock will be judged by the agricultural industry solely on total economic merit. Assessing the cost-benefit ratios of a project is a complicated process at best; assessing the economics of projects directed toward solving problems of human disease, which involve moral considerations, adds to the complexity. However, the economics of even those types of projects can and should be evaluated. Cost assessment of the merits of a given strategy for improving livestock traits is more straightforward. With current technology, the cost of producing transgenic cows is so great that very few organizations have been willing to invest in experiments designed to assess the technical feasibility of altering cow's milk to create new food products. Without that information, it may be premature to speculate on other, potentially larger, project costs that may be incurred to alter marketing strategies and processing systems and to meet regulatory requirements for the new animal products.

Time is the greatest resource expended in a transgenic cattle project. Using techniques now considered standard, 7 to 8 yr are needed to produce a milking herd of transgenic dairy cattle. Policy makers should not view this situation as a justification to abandon the development of these technologies but, rather, as an indication that additional research resources should be made available to overcome the impediments that hinder progress.

Before gene modifications relating to the mammary gland are discussed in detail, it is appropriate to consider the current status of transgenic animal technology. This article addresses some of the hurdles that continue to impede application of this technology from solving agricultural problems.

TRANSGENIC ANIMAL MODEL SYSTEM

Most of the goals currently proposed for genetically altering milk composition rely on gain of function strategies (introduction of a new gene) by means of pronuclear microinjection. However, eliminating or reducing the concentration of specific proteins in milk can also be achieved, at least in theory, by adding new genetic information. By introducing ribozyme or anti-sense genes, translation of specific milk protein genes can be blocked or reduced, thus reducing their protein concentration in milk.

A more widely accepted means of achieving loss of function (eliminating or altering gene function) is through use of gene "knock-out" technology that is dependent on embryonic stems cell. Although outside the scope of this review, development of embryonic stem cells of domestic livestock is being pursued by a number of groups, and embryo-derived pluripotent cell lines have been reported for the pig (81) and cow (71, 73); germline transmission from these cells has not been reported. Recently, lambs were produced following nuclear transfer from an established cell line derived from sheep embryos (31). It remains to be demonstrated whether these cells can be transfected with new genes and selected prior to nuclear transfer and whether such cells can be isolated from other livestock species.

Production of transgenic animals by pronuclear microinjection is practiced today essentially as described in the pioneering first report of Gordon et al. (27), as further characterized by Brinster et al. (11), and as adapted to livestock by our laboratory (33, 78). Table 1 lists the milestones toward produc-

tion of transgenic bioreactors. Interestingly, although the technique has changed little, the definition of the word "transgenic" has taken on new meanings. Jon Gordon and Frank Ruddle originally proposed the term to describe animals in which new genetic material had been introduced by pronuclear microinjection (27). The term has been broadened considerably to include genetically engineered plants, animals produced with the aid of stem cells, and animals that have been subjected to in vivo somatic cell engineering (injection of DNA directly into living animals).

Efficiency of Producing Transgenic Animals

The efficiency of producing transgenic animals is low. A compilation of data from several laboratories indicated that about 1 transgenic animal was produced per 40 mouse eggs injected, and the efficiency for sheep, goats, and cattle was much lower, requiring approximately 110, 90, and 1600 egg injections per transgenic animal, respectively (77). Furthermore, only about 50% of transgenic offspring express their transgene. To those working with mice, low efficiency is not of particular concern, but low efficiency is a major impediment to those attempting to produce transgenic livestock. Three parameters account for the low efficiency of the process: embryo survival, gene integration rate, and transgene behavior. In both livestock species and laboratory animals, about 15% of microinjected, transferred embryos survive to term (77). However, gene integration frequency, as measured by proportion of animals born that are transgenic, is much lower for livestock species than for laboratory species (8, 33). That difference in integration rate may be indicative of important biological differences between the zygotes of these species.

Transgene Integration Frequency

The genetic diversity of livestock species may affect the low integration frequency observed. Laboratory animals are derived from highly inbred lines, and investigators often chose to use specific strains for which embryos can be cultured easily. Scientists working with livestock embryos do not have the same inbred resources. However, there are obvious examples in the livestock industry of selection of animals based on gamete quality. One example is the AI industry in which selection of bulls is based not only on genetic merit but also on the freezability of the sperm. Very few researchers have the resources to perform the same kind of selection on embryo donors.

Another possible cause for the low rate of transgene integration may be related to procedural differences between microinjection of zygotes of livestock and laboratory animals. Livestock eggs are more challenging to microinject than are eggs of mouse, rabbit, or rat. Cow and sow eggs must be centrifuged before microinjection. Although little evidence exists that embryo survival is compromised by centrifugation (78), the procedure may somehow influence integration rate. Within livestock species, the lower integration rate that has been observed for cattle embryos may be partially attributable to the increased difficulty of microinjecting cow eggs. However, that difficulty is not likely to account for the reduction in integration efficiency between cattle and other livestock species.

It is also possible that transgenes become integrated at similar rates but that the development of transgenic livestock embryos and fetuses is disproportionately compromised by transgenes. However, the similar and poor survival rate of both laboratory and livestock embryos does not support that hypothesis. With the data available, it is not possible to determine to what extent susceptibility to transgenes or expression of transgenes accounts for poor livestock embryo survival. Those are probably not a major component (36).

A more compelling argument could be made for an association of integration failure with the inappropriate timing of microinjection. It is well known that transfection of cells in culture is most efficient in dividing populations. Inference from that observation is that DNA replication is required for integration of foreign genes into the genome (4), in which case, the timing of pronuclear microinjection should be synchronized with onset of the DNA S-phase (synthesis phase) of the first cell cycle to ensure the maximum likelihood of an integration event. Mouse eggs are microinjected at about 8 h postinsemination, resulting in DNA being introduced into zygotes during the beginning of S-phase (44). Cow eggs are injected toward the end of the S-phase (23, 24, 40), possibly reducing the probability of an integration event. Pronuclei in cattle zygotes form well before the normal timing of microinjection. However, the normal timing of microinjection is restricted to the time that pronuclei can be visualized in a nondestructive manner (differential interference contrast microscopy). At earlier times, pronuclei cannot be found, even though they are present. Therefore, it would seem to be impossible to microinject transgenes before DNA synthesis is nearly completed in cow eggs. Ex-

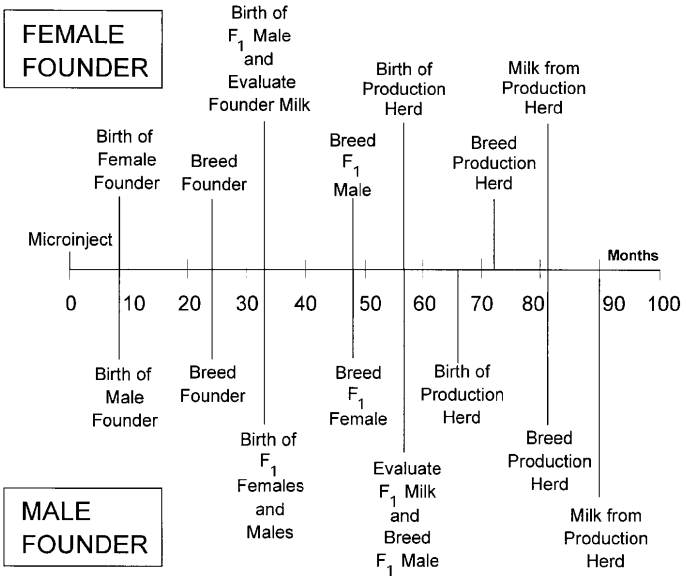

Figure 1. Time line (months) from microinjection to lactation of cows in a transgenic dairy herd arising from a male or female founder. It is assumed that the gestation period is 9 mo, that both males and females reach sexual maturity at 15 mo of age, that the production herd is sired (AI) by a single F_1 male from a line known to express the transgene, and that the first calf of the female founder is male.

perimental evidence is lacking to support the influence of microinjection timing on the frequency of transgene integration, and such evidence may be difficult to achieve. Efforts to inject in vitro fertilized bovine zygotes earlier than 13 h after insemination have failed because of difficulties in visualizing pronuclei and because attempts to manipulate the timing of events during the first cell cycle have been inconclusive.

Transgene Expression

Characteristics of transgene expression in transgenic goats, mice, pigs, rabbits, rats, and sheep appear to be similar. Although data are insufficient to characterize transgene expression for transgenic cattle, there is no reason to expect that transgenes behave differently in that species. Transgenes appear to integrate randomly in the genome, and approximately half of transgenic animal lines express their transgenes, although some specific transgenes are expressed in a higher proportion of transgenic animals (16, 29). Even in lines that express their transgene, transgene expression is often inappropriate, occurring in unintended tissues (ectopic expression) or at developmentally incorrect times. These aberrant expression patterns or lack of expression have been attributed to the "position effect", which suggests that neighboring genes or heterochromatin regions can override the control of transgenes. Addition of matrix attachment region sequences or genetic boundary elements to transgene constructs may overcome the position effect in lines that would otherwise carry nonfunctional transgenes (52).

Transgene Design and Evaluation

The selection of appropriate regulatory regions, coding sequence type (cDNA versus genomic), introns, and polyadenylation signals for design of mammary specific fusion genes has been severely hindered by the lack of a rapid screening technique. Fusion genes containing promoter regions that are specific to the lactating mammary gland can only be evaluated in lactating mammary epithelial cells. Unfortunately, a transfectable, fully functional system for culturing mammary cells has not been developed. Thus, the labor-intensive and time-consuming transgenic mouse model is viewed as the only reliable technique for evaluation of gene constructs. Even with the relatively short gestation period of the mouse, meaningful evaluation of a lactation-specific construct in F_1 transgenic offspring requires a minimum of 6 to 12 mo. As an initial screening tool, we are developing an in situ transfection technique based on jet injection of naked DNA into lactating mammary glands (25). At 2 d after jet injection of plasmid DNA containing the

human growth hormone (**hGH**) gene, driven by the human cytomegalovirus promoter-enhancer region into mammary glands of lactating sheep, transgene expression was sufficient to be detected by Northern blot analysis (41). We are now using this technique to compare the potencies of various mammary-specific promoter regions in directing expression of the hGH gene (42). Expression is being assessed by both Northern blot analysis and by measurement of hGH protein in tissue extracts. Using the ovine β-LG promoter, up to 40 ng/ml of hGH protein has been detected in the extracts of mammary tissue obtained 48 h postinjection. We are confident that this approach will speed the process of transgene evaluation, serving as a preliminary screening technique prior to the transgenic mouse model, which is much more labor intensive. Finally, because the gene product (protein) can be detected after jet injection of the gene construct, evaluation of posttranslational capabilities of the target tissue in the target species is possible.

Time Requirements for Transgenic Animal Projects

Time is by far the greatest resource expended in transgenic livestock projects, as is clearly shown in projects in which the transgene is designed to express only in the lactating mammary gland. In such projects, the minimum time from microinjection to evaluation of milk is equal to twice the gestation length of the target species plus the time from birth to puberty. Once a useful line has been identified, the next step is to collect semen in order to generate a production herd. The time required to achieve these milestones using standard techniques is presented in Figure 1. Clearly, there is a need to develop strategies that shorten the 7 yr to production.

A number of strategies have been demonstrated or proposed to reduce the time that is required to identify and evaluate transgenic dairy animals as well as the time that is required to expand successful lines (7). For example, expression of a lactation-specific transgene has been detected in milk obtained by artificial induction of lactation in female and male goats (21). More recently, expression of a fusion gene composed of the bovine α_{S1}-CN promoter driving the hGH gene was detected by mammary biopsies obtained 24 h after birth of both male and female rats (37). If such a strategy were proved to reflect transgene expression in adults, then semen from a transgenic bull, collected at approximately 15 mo of age (2 yr following microinjection), could be used to generate a herd producing genetically modified milk in approximately 3 yr—about 5 yr from the time of microinjection.

TRANSGENIC ANIMAL BIOREACTORS

A number of companies have been formed within the last decade specifically to exploit transgenic technology. This new industry has been created based on the assumption that production of pharmaceuticals in transgenic animals is more cost effective than production by more conventional means. The US market for bioreactor products thus far identified exceeds $3 billion/yr (Table 2). All of the products listed in Table 2 are currently derived from human blood, which may account for the fact that the American Red Cross Blood Derivatives Laboratory is one of the leaders in the bioreactor field.

Advantages of Transgenic Animal Bioreactors

Almost any living organism, or part thereof, that can be cultivated can serve as a bioreactor. Bacteria, yeast, insect cells, mammalian cells in culture, plants, and chicken eggs are all potential competing produc-

TABLE 2. Estimated annual US requirements and costs of some potential bioreactor products.[1]

	Pharmaceutical					
Item	F VIII[2]	F IX[3]	Protein C	AT III[4]	Fibrinogen	Albumin[5]
Estimated quantity needed, kg	0.3	4	10	21	150	315×10^3
Current cost per gram, $	2,900,000	40,000	10,000	7000	1000	3.56
Annual market, $ $\times 10^6$	882	160	100	150	150	1120

[1]Information from William Drohan and Henryk Lubon, American Red Cross (46, 56).

[2]Blood coagulating factor VIII.

[3]Blood coagulating factor IX.

[4]Antithrombin III.

[5]Human serum albumin.

tion systems. Each system has specific advantages and disadvantages. In general, prokaryotic systems and plants can be genetically engineered and propagated rapidly at relatively low cost but lack the mechanisms, or possess the wrong machinery, to perform some of the critical posttranslational modifications (e.g., signal peptide cleavage, glycosylation, amidation, acetylation, carboxylation, and phosphorylation) that are required by complex mammalian proteins (38). Systems for baculovirus-insect cell expression (47) and stable transfected mammalian cells have the capacity to perform authentic posttranslational modifications, but yields in those systems are often an order of magnitude lower than those already achieved in transgenic animal bioreactors.

Advantages of the Transgenic Mammary Gland

The mammary gland is a prodigious production system that is capable of generating between 23 g (dairy cattle) and 205 g (rat) of protein/kg of body weight during peak lactation (55). Milk is clearly the easiest body fluid to collect, especially from ruminants; even pigs can be milked mechanically (30). The ample production capacity of the mammary gland, coupled with the relative ease of harvesting milk in a noninvasive manner, recommends the mammary gland as the organ of choice for producing pharmaceutical products from animals.

Another often cited advantage of producing biologically active products using the mammary gland is the isolation of the mammary gland from the circulatory system. It is argued that bioreactor animals would be protected from the potentially untoward effects of biologically active compounds because those compounds would be sequestered in the mammary gland and therefore would be unavailable to the circulatory system. However, endogenous milk proteins are indeed found in the circulation in cattle, especially during late gestation and at parturition (51), and transgenes and milk protein genes are transiently expressed during estrus, even in virgin mice (64). Therefore, to safeguard bioreactor animals, it may be appropriate to consider designing gene constructs in such a way that their product is converted to an active form after it is isolated from milk.

A potential hurdle to the success of mammary gland bioreactors lies in the ability of the alveolar epithelium to provide appropriate posttranslational modifications such as cleavage of propeptides or signal peptides, N- or O-glycosylation, and γ-carboxylation. Lack of faithful posttranslational modification of a protein is assumed to affect its biological activity. Furthermore, carbohydrate residues of glycoproteins can serve as antigens, can also influence secretion of a protein, and can affect the half-life of a protein in the circulation (39).

It has recently been shown that, when transgenes for interferon-γ were expressed in ovary cells of Chinese hamsters, Sf9 insect cells infected with baculovirus, and mammary glands of transgenic mice, N-glycosylation patterns (sites glycosylated) and composition of the sugar residues differed significantly (39). Glycosylation patterns of human Protein C produced in the mammary glands of transgenic mice (20) or transgenic pigs (54) have been demonstrated to differ from each other and from Protein C isolated from human serum. No additional data are available to assess the significance of these observations on the biological activity of this protein or on the production of other pharmaceuticals in milk. However, at least one prominent laboratory in the bioreactor field is beginning to address the issue by creating transgenic mice that contain two transgenes directed toward the mammary gland, one for the protein of interest and the other to increase the posttranslational modification capabilities of the gland (19).

Production Capacity of Transgenic Mammary Glands

Transgene production capacity is difficult to predict with any certainty. However, from the growing list of studies on transgenic animals in which a milk protein promoter has been used to direct expression of pharmaceutical (Table 3) or milk protein (Table 4) into milk, reasonable production would be at least 1 mg/ml.

The bovine is the target species for transgenic projects aimed at modifying milk for the dairy industry. For bioreactor projects, the target species will be determined on product demand. At an assumed production level of 1 mg/ml, one can calculate the number of animals that would be required to produce some of the proposed pharmaceutical bioreactor products. The results of those calculations are presented in Table 5. On first inspection, it seems unreasonable to think that an organization would consider generating the more than 27,000 rabbits necessary to produce 150 kg of fibrinogen. The labor that is required to maintain and milk those animals would be enormous, especially in light of the fact that 17 cows might be capable of producing all of the fibrinogen required to satisfy current world needs.

TABLE 3. Summary of transgenic animal studies in which a milk protein promoter was used to direct expression of a milk protein into milk.

Transgenic species	Coding sequence		Promoter region		Protein in milk[1]	Reference
	Gene	Source	Gene	Source		
Murine	α-LA	Bovine	α-LA	Bovine	(mg/ml)	(5, 70) (76)
Murine	α-LA	Caprine	α-LA	Caprine	mg	(70)
Murine	α-LA	Guinea pig	α-LA	Guinea pig	ND[2]	(50)
Murine	β-LG	Ovine	β-LG	Ovine	mg	(35, 68)
Murine	α_{S1}-CN	Bovine	α_{S1}-CN	Bovine	ND	(15)
Murine	β-CN	Bovine	β-CN	Bovine	mg	(62)
Murine	β-CN	Bovine	α-LA	Bovine	mg	(6)
Murine	α-CN	Caprine	β-CN	Caprine	mg	(58, 63)
Murine	β-CN	Rat	β-CN	Rat	mg	(45)
Murine	κ-CN	Bovine	β-CN	Caprine	mg	(31)
Murine	κ-CN	Caprine	β-CN	Caprine	mg	(57)
Murine	Lactoferrin	Human	α_{s1}-CN	Bovine	mg	(59)
Murine	Lysozyme	Human	α_{s1}-CN	Bovine	μg	(48)
Murine	WAP[3]	Murine	WAP	Murine	mg	(13, 52)
Murine	WAP	Rat	WAP	Rat	mg	(2, 16)
Porcine	WAP	Murine	WAP	Mouse	mg	(66, 79)
Ovine	WAP	Murine	WAP	Mouse	μg	(80)
Bovine	Lactoferrin	Human	α_{s1}-CN	Bovine	ND	(43)

[1]Precision of protein determinations varied between publications. Therefore, only the order of magnitude of concentration is given for comparison purposes.

[2]Not determined.

[3]Whey acidic protein.

TABLE 4. Summary of transgenic animal studies in which a milk protein promoter was used to direct expression of a pharmaceutical protein into milk.

Transgenic species	Coding sequence		Promoter region		Protein in milk[1]	Reference
	Gene	Source	Gene	Source		
Murine	α_1-Antitrypsin	Murine	WAP[2]	Rabbit	(mg/ml)	(3)
Murine	α_1-Antitrypsin	Human	β-LG	Ovine	mg	(1)
Murine	β-Interferon	Human	WAP	Murine	ND[3]	(65)
Murine	γ-Interferon	Human	β-LG	Ovine	ng	(18)
Murine	CFTR[4]	Human	β-CN	Caprine	μg	(17)
Murine	Factor IX	Human	β-LG	Ovine	μg	(85)
Murine	Protein C	Human	WAP	Murine	ng	(75)
Murine	Serum albumin	Human	β-LG	Ovine	mg	(67)
Murine	Superoxide dismutase	Human	β-LG	Ovine	ng	(34)
Murine	Superoxide dismutase	Human	WAP	Murine	mg	(34)
Murine	t-PA[5]	Human	WAP	Murine	ng	(28)
Murine	t-PA	Human	α_{s1}-CN	Bovine	μg	(61)
Murine	Trophoblastin	Ovine	α-LA	Bovine	μg	(72)
Murine	Urokinase	Human	α_{s1}-CN	Bovine	mg	(53)
Rabbit	Interleukin-2	Human	β-CN	Rabbit	ng	(12)
Rabbit	t-PA	Human	α_{s1}-CN	Bovine	μg	(61)
Porcine	Protein C	Human	WAP	Murine	mg	(74)
Ovine	α_1-Antitrypsin	Human	β-LG	Ovine	mg	(83)
Ovine	Factor IX	Human	β-LG	Ovine	ND	(69)
Caprine	t-PA	Human	WAP	Murine	μg	(22)

[1]Precision of protein determinations varied between publications. Therefore, only the order of magnitude of concentration is given for comparison purposes.

[2]Whey acid protein.

[3]Not determined.

[4]Cystic fibrosis transmembrane receptor.

[5]Tissue plasminogen activator.

However, the required number of rabbits could be produced in 3 to 4 yr by using homozygous males and AI, but 7 to 8 yr would be needed to produce the 17 cows. This comparison is exaggerated but points out the need to consider generation interval as well as production capacity when choosing a species for a bioreactor project.

The data presented in Table 5, admittedly based on preliminary findings, bode well for the practical ability for genetic engineering of mammary glands to produce commercially useful concentrations of foreign protein. Nevertheless, the amount of transgene proteins produced are lower than those of endogenous proteins for all species with the possible exception of sheep. Are the relatively modest concentrations of transgene proteins that have been observed the result of inept transgene design, or are mammary glands already producing close to their maximum capacity? Will milk protein genes have to be knocked out to provide additional capacity for transgene proteins? Will higher transgene product production levels be disruptive to mammary gland function as they were in one study (6)? These questions cannot be answered at this time.

MODIFICATION OF MILK TO IMPROVE NUTRITION AND PROCESSING

As mentioned at the outset, a variety of ways have been suggested to modify milk to improve its nutritional quality and to improve the efficiency of manufacturing milk products such as cheese, ice cream, and yogurt (Table 6). Unfortunately, this area of investigation has received less attention in laboratories than it has in review articles.

The limited efforts in this area of research, compared with research in pharmaceutical production, may reflect the perceived rate of financial return on food products compared with the return on biomedical products. However, a less obvious factor may also have influenced the differences in emphasis of the two fields of genetic engineering. A significant segment of the pharmaceutical industry has embraced the transgenic bioreactor as an approach to making drugs, but little evidence exists that the dairy industry has been as enthusiastic about modifying the type of milk that cows produce. The pharmaceutical industry has a history of obtaining its products from a variety of sources—extracting drugs from plants, synthesizing drugs from chemical constituents, and isolating drugs from animal and human tissues. Isolating these products from the milk of genetically engineered animals does not require a significant change in mind set. However, the prospect of creating cows that produce specialized milk may have momentous consequences for the structure of the dairy industry.

The goal of the dairy industry, at least in the US, has been to create an efficient, healthy cow producing copious amounts of milk that can serve all the needs of the industry. Genetic engineering offers the opportunity for a paradigm shift, a reshaping of the industry from the producers to the processing plants. Dairy producers have the opportunity to choose to produce high protein milk; milk destined for cheese manufacturing that has accelerated curd clotting time; milk containing nutraceuticals, orally administered biologics that provide a health benefit; or a replacement for infant formula. Such a scenario would be a radical change for the dairy industry. But this scenario is by no means without precedent in agricultural production systems. One only has to look to grain, fruit, and vegetable crops to see numerous examples of varieties that have been propagated to fill or create specific niche markets. Whether such a paradigm shift will

TABLE 5. Estimated number of transgenic animals needed to satisfy the annual US market for selected pharmaceuticals.[1]

	Pharmaceutical					
Species	F VIII[2]	F IX[3]	Protein C	AT III[4]	Fibrinogen	Albumin[5]
Rabbit	54	714	1785	3750	27×10^3	56×10^6
Porcine	1	10	25	53	380	800×10^3
Ovine	1	13	33	70	500	1050×10^3
Caprine	1	7	17	35	250	525×10^3
Bovine	1	1	2	3	17	35×10^3

[1]Based on estimated quantity needed (Table 2) and a transgene protein production of 1 g/L.

[2]Blood coagulating factor VIII.

[3]Blood coagulating factor IX.

[4]Antithrombin III.

[5]Human serum albumin.

TABLE 6. Some proposed modifications of milk constituents.[1]

Change	Consequence
Increase α- and β-CN	Enhanced curd firmness for cheese making, improved thermal stability, and increased calcium content.
Increase phosphorylation sites in caseins	Increased calcium content and improved emulsification.
Introduce proteolytic sites in caseins	Increased rate of textural development (improved cheese ripening).
Increase κ-CN concentration	Enhanced stability of casein aggregates, decreased micelle size, and decreased gelation and coagulation.
Eliminate β-LG	Decreased high temperature gelation, improved digestibility, decreased allergenic response, and decreased primary source of cysteine in milk.
Decrease α-LA	Decreased lactose, increased market potential of fluid milk, decreased ice crystal formation, and compromised osmotic regulation of mammary gland.
Add human lactoferrin	Enhanced iron absorption and protected against gut infections.
Add proteolytic sites to κ-CN	Increased rate of cheese ripening.
Decrease expression of acetyl-CoA carboxylase	Decreased fat content, improved nutritional quality, and reduced milk production costs.
Express Ig genes	Protected against pathogens such as salmonella and listeria.
Replace bovine milk proteins genes with human equivalents	Mimicked human breast milk.

[1]Adapted (14, 38, 84).

sweep through the entire dairy industry or become a subset of the industry remains to be seen. There is, however, little question that products of genetic engineering will become part of the dairy industry in the next century.

Many of the proposed changes in milk structure are listed in Table 6. It is readily apparent that a number of ways may be used to achieve the same or similar goals. Clearly, altering the characteristics of one component to enhance a particular processing feature may make milk unsuitable for other uses. During the next decade, dairy scientists must determine which approach is most efficacious to achieve the desired goal.

Gibson (26) recently published a thorough analysis of the economic potential of altering milk fat composition and pointed out several potential limitations to that strategy. One consequence of altering milk fat composition would be that increasing the proportion of unsaturated fats (and as a consequence decreasing saturated fats) would increase the melting point of milk, which could impair whipping of cream and increase the hardness of butter. Furthermore, if the alterations resulted in increased free fatty acids, milk rancidity would likely increase. Gibson (26) concluded that the interactive consequences of altering fat content of milk on processing of other milk products would not be economically advantageous to large segments of the dairy industry, but would only be suitable for niche markets.

However, if the goal is to decrease total fat content rather than to alter the ratios of various fat components, a different economic picture emerges. On average, dairy cows in the US produce milk that contains 3.8% fat. Approximately 50% of that fat is synthesized in the mammary gland. Therefore, if that synthetic machinery in the mammary gland could be disrupted, milk containing 2.0% fat (a 40% reduction) might be achievable (84).

Bremel et al. (9) have suggested that de novo fat synthesis might be reduced by blocking expression of the acetyl-coenzyme A (**CoA**) carboxylase gene through stem cell ("knockout"), antisense, or ribozyme technology. A recent study (32) confirmed the potential of this concept by transfecting preadipocyte cells with a ribozyme gene directed against acetyl-CoA carboxylase RNA. Acetyl-CoA carboxylase RNA was reduced compared with nonribozyme-expressing cells. The decrease in acetyl-CoA carboxylase mRNA was associated with a significant decrease in activity of acetyl-CoA carboxylase enzyme, and the rate of fatty acid synthesis fell to about 30 to 70% that of controls. This strategy would also have collateral consequences, but might be very cost effective.

Fats are energetically expensive molecules to make, and reducing the fat content of milk to 2.0% could reduce feed energy requirements. Typical dairy rations consist of about 60% forage and 40% concentrate. The cost of forage accounts for about 40% of the cost of feed, and the grain component accounts for about 50% of the feed cost. Engineering of cows to produce 2.0% fat milk would allow the forage component of rations to be increased to 83% (84). The consequence of that change in feed composition would be a reduction of 22% in feed cost per kilogram of milk. Notwithstanding significant savings to the dairy producer, further ramifications could have a positive environmental impact. The amount of forage production would have to be increased by 33%, but the amount of corn produced for dairy rations could be decreased by 50%. Based on corn and forage yields at Beltsville, an acre of land could be taken out of corn production for every 4 cows engineered to produce 2.0% fat milk. Over 2 million acres used for grain production could be reclaimed if all 10 million dairy cows in the US were genetically engineered to produce 2% fat milk. Admittedly, this occurrence is unlikely but demonstrates the potential impact that genetic engineering of dairy cattle could have on other aspects of agriculture.

CONCLUSIONS

The most easily identified constraint impeding progress in this field is the difficulty in producing useful transgenic animals. As mentioned earlier, low rates of embryo survival and transgene integration have clearly been documented. Because of those two factors, producing a transgenic sheep or goat can easily cost $60,000, and producing a transgenic cow or bull can exceed $300,000. The situation is further exacerbated by the unpredictable behavior of fusion gene constructs. Currently, no foolproof method to assess the function of a transgene exists, other than to use it to make transgenic animals of the species of interest. Most organizations test transgene constructs in mice before introducing them into livestock. However, transgenic mice have proved to be poor predictors of transgene function in another species. A way must be found to evaluate transgene constructs accurately in the tissue of the animal of choice without requiring the investment of time and money to produce transgenic livestock. Increased efficiency of producing transgenic animals not only would have the obvious benefit of reducing costs, but also would encourage participation in this field by investigators who cannot currently afford to use this technology. The participation of other researchers is necessary, especially in the area of modifying milk for postharvesting processing purposes because of the great deal of expertise and research that is required to link the fields of transgenic animal production and dairy food science.

Transgenic animal bioreactor organizations have demonstrated, mostly through empirical studies, that almost any desired protein can be produced in mammary glands. This field of research has now reached the stage of maturity at which the focus of attention will shift to concerns of posttranslational modifications of those proteins, efficacious purification schemes, and product safety. Sixteen years ago, transgenic animal technology was invented, and, since then, an industry has formed to exploit that technology. In the next 16 yr, products created by that technology will likely be in the hands of consumers.

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